INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Chiral HPLC Method Development And Validation For The Estimation Of S-Viloxazine And FD Characterization By MS

Shaik Mastanamma*[1], Chunduri Akhila[2], M. S. Dhandapani[3]

[1]*Department Of Pharmaceutical Analysis, Acharya Nagarjuna University College Of Pharmaceutical Sciences*
[2]*Department Of Pharmaceutical Analysis, Acharya Nagarjuna University College Of Pharmaceutical Sciences*
[3]*Department Of Pharmaceutical Analysis, Acharya Nagarjuna University College Of Pharmaceutical Sciences*

ARTICLE INFO

Received: 17 Aug 2024
Accepted: 21 Aug 2024
Published: 25 Aug 2024

Keywords:
chiral HPLC, s-viloxazine, enantiomers, FD characterization.

DOI:
10.5281/zenodo.13371310

ABSTRACT

In the present work, aim to develop and validate a new High-Performance Liquid Chromatography (HPLC) method performing chiral separation for estimation of s-viloxazine and foerced degradation characterization. Optimization led to the development of an enantioselective method for rapid detection and quantitative analysis, with baseline resolution at purity levels in a simple hplc s-viloxazine degradation products utilizing chiral stationary phase. The parameters like mobile phase composition, column temperature and flow rate were systematically optimized for the best separation of peaks with good resolution. Quaternary gradient pump of e2695 series with auto sampler injector in which 10µl was injected and eluted using mobile phase consisting Methanol: n-hexane, IPA (30:50; 20 v/v) at a flow rate of 1ml/min, chiralpack IM, 250x10mm,(C-18 chrial column)5 µm) was used for sample separation, and UV detector set at λmax264.6nm were employed as analytical tools for the quantification analysis. The method developed was found to be both specific, linear as well as precise accurate and robust. The drug was stressed under different stress condition like acid, alkali, peroxide, reduction, photolytic, hydrolysis and thermal degradation. However, stress degradation studies were conducted to generate 4 major degradation products which are DP-1, Dp-2, Dp-3, and Dp-4 respectively. The formed degradation products are subjected to mass characterization. The results for the chosen drug's recoveries were found to be within acceptable ranges (98–102%).

INTRODUCTION

2-[(2-ethoxyphenoxy) methyl] morpholine is s-viloxazine. The drug s- viloxazine belongs to a group of drugs known as selective norepinephrine reuptake inhibitors. s- Viloxazine is available at these dosages of 100–200 mg and is sold under the brand name Qelbree. Literature review reveals the different methods to estimate the concentration of

***Corresponding Author:** Shaik Mastanamma
Address: *Department Of Pharmaceutical Analysis, Acharya Nagarjuna University College Of Pharmaceutical Sciences*
Email ✉: masthanamma.sk@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

s- viloxazine with or without any combination in biological fluids. However, there is no literature relating to the determination and degradation product characterization of s- viloxazine. The present study is intended to establish a method for the determination and degradation product characterization of s- viloxazine by LC-MS.

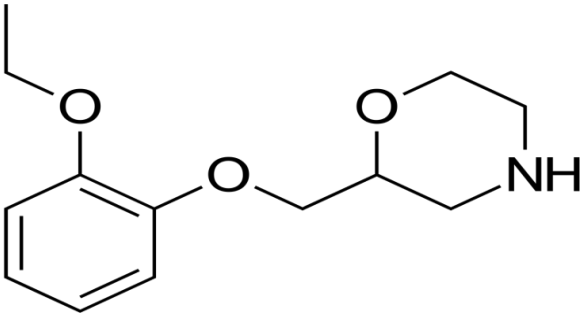


Fig no; 1 Molecular structure of s- Viloxazine

EXPERIMENTALWORK

Chiral- HPLC Method Development for s-Viloxazine

Instrument:

The Waters Alliance e-2695 type HPLC was utilized for analysis, equipped with a column, auto sampler, and degasser. The SCIEX QTRAP 5500 mass spectrometer, which has an electron spray ionization interface, was connected to the HPLC system. The chromatogram's data was interpreted using SCIEX software. pH meter- Eutech, UV/ vis spectrometer –UV-1700, Ultrasonicator- UCA701- Unichrome were used.

Reagents& Chemicals

TableNo.1: List of chemicals used in HPLC Method

S. No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water(Milli Q)	HPLC	Inhouseproduction
3.	Isopropyl alcohol	HPLC	Analyticalreagents
4.	Methanol	HPLC	Rankem
5.	n-hexane	HPLC	Analytical Reagent

Drug Samples:

Viloxazine(RS), s-viloxazine was obtained as a gift sample from Shreeicon laboratories, Vijayawada, India.

Preparation of solutions:

Preparation of Racemic Mixture Standard stock **solution:**

Accurately weigh and transfer 50 mg of the s-viloxazine working standard into a 10 ml dry volumetric flask. In order to completely dissolve it, sonicate, add diluent, and use the same solvent to raise the volume to the required level. (Ordered by stocks). Proceed by transferring 1 milliliter of the previously described solution into a 10-milliliter volumetric flask and incorporating diluents to yield the desired result.

Preparation of s- viloxazine standard stock solution

Accurately weigh and move 50 mg of the s-Viloxazine working standard into a 10-milliliter volumetric flask that has been thoroughly cleaned and dried. Use the same diluent to get the volume up to the desired level after adding the diluent and sonicating to completely dissolve it.

Preparation of Impurity Stock Solution:

Weigh out exactly 5 milligrams of s-Viloxazine impurity-1 that has been added to a volumetric flask with a capacity of 10 milliliters. After adding 7 milliliters of diluent and sonicating it to dissolve it fully, use the same diluent to adjust the volume. Transfer 1 milliliter of the previously described solution into a 10-milliliter volumetric flask and use diluents to correct the mark.

Preparation of Spiked standard solution:

Fill a 10 ml volumetric flask with 1 ml each of the standard stock solution for s-viloxazine and the

impurity stock solution. Add diluents to the appropriate level. Use filter paper with a 0.45µm pore size for filtration. (5ppm of Imp-1 and 500ppm of s-Violazine)

Preparation of Mobile Phase:

Methanol, n-Hexane, and IPA were combined in a ratio of 30:50:20 to create the mobile phase. To get rid of any contaminants that might have affected the final chromatogram, it was filtered via a 0.45µ membrane filter for filtration.

Determination of Working Wavelength (λmax):

The drug's isobestic wavelength was estimated using this method. The wavelength at which the molecular absorbance of substances that are interconvertible is the same is known as the isobestic point. Thus, this wavelength was utilized to estimate the medication precisely. The drug's solution's maximum absorption wavelength, as well as any impurities present in the mixture of methanol: n-Hexane: IPA (30:50:20) was scanned against methanol: n-Hexane: IPA (30:50:20) as a blank using a PDA detector in the 200–400 nm wavelength range. The isobestic point on the absorption curve is located at 264.6 nm. Thus, using the HPLC chromatographic procedure, a detector wavelength of 264.6 nm was used.

Chromatographic conditions

Column:

Chiralpack IM, 250x10mm, 5µm, SFC Semi prep

Mobile Phase: Methanol and n-Hexane and IPA (30:50:20)

Temperature:

ambient temperature

Injection volume:

10 µl

Flow rate:

1 ml/min

Detection:

264.6 nm

Run time:

10 min

Mass spectrometer conditions:-

The mass spectrometer was managed in positive ion electron spray ionization interface mode.Multiplereactionsmonitoringmodehasbeenappliedtoquantifytheviloxazine.Workingparametershavebeen set as follows:

- Collisionenergy:14V
- Ion spray voltage: 5500V
- Sourcetemperature:550oC
- Dryinggastemperature:120-250°C
- Collision gas: nitrogen
- Dryinggasflowstream:5mL/min
- Declustering potential: 40 V
- Entrancepotential:10V
- Exit Potential: 7 V
- Dwelltime:1sec

DEGRADATIONSTUDIES:

Acid degradation:

Pipette1ml of s- viloxazine stock solution into a10ml volumetric flask and add 1ml of 1N HCl. The volumetric flask was then maintained at 60°C for an hour, after which it was neutralized with 1N NaOH and diluted with diluent to yield 10 ml.

Alkali degradation:

Pipette1ml of s- viloxazine stock solution into a10ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1N HCl and make up to 10ml with diluent.

Thermal degradation

s- Viloxazine working standard was taken in petridish and kept in Hot air oven at 1050 C for 3hours. Then the sample was taken and diluted with diluents and analysed.

Peroxide degradation

The pipette 1 milliliter of the s- viloxazine stock solution and 1 milliliter of 3 percent w/v hydrogen peroxide were added to a 10 milliliter volumetric flask, and the volume was then diluted with diluent until the target level was reached. The volumetric flask was then maintained at 60°C for an additional

hour. After that, the volumetric flask was left for fifteen minutes to stand at room temperature.

Reduction degradation

The pipette 10 milliliter volumetric flasks were filled with 1 milliliter of s - viloxazine stock solution, 1 milliliter of 10% sodium bisulphate, and the volume was increased with diluent to the necessary volume. The volumetric flask was then kept at 60°C for an hour. The volumetric flask was then allowed to stand at room temperature for fifteen minutes.

Photolytic degradation

Viloxazine (S-isomer) working standard was placed in Photo stability chamber for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Hydrolysis degradation

The pipette A 10 ml volumetric flask was filled with 1 ml of s- viloxazine stock solution, 1 ml of HPLC grade water, and diluent was used to raise the volume to the necessary volume. After that, the volumetric flask was kept at 60°C for an hour. The volumetric flask was then allowed to stand at room temperature for fifteen minutes. All the stressed samples was transferred to vials after filtration for HPLC analysis

RESULTS AND DISCUSSION

The chiral-HPLC method was designed to estimate s-Viloxazine and its impurities simultaneously using a Chiralpack IM, 250x10mm, 5µm, SFC Semi prep column. The mobile phase is made up of methanol, n-hexane, and IPA in the ratio of 30:50:20 v/v, with a flow rate of 1.0 ml/min. The ambient column temperature was maintained and the detection was performed at 264.6 nm.

Figure-2 Chromatogram of s- Viloxazine Racemic Mixture

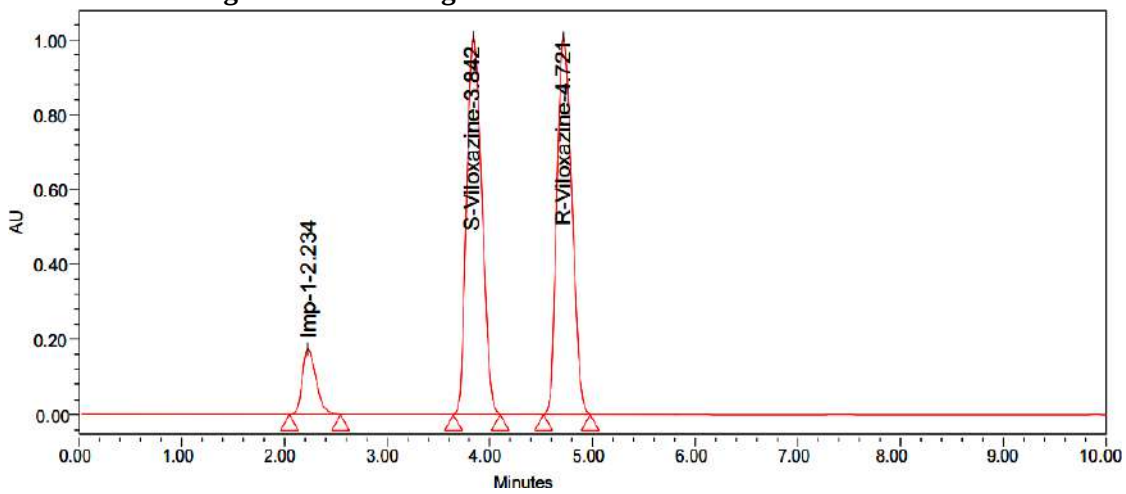
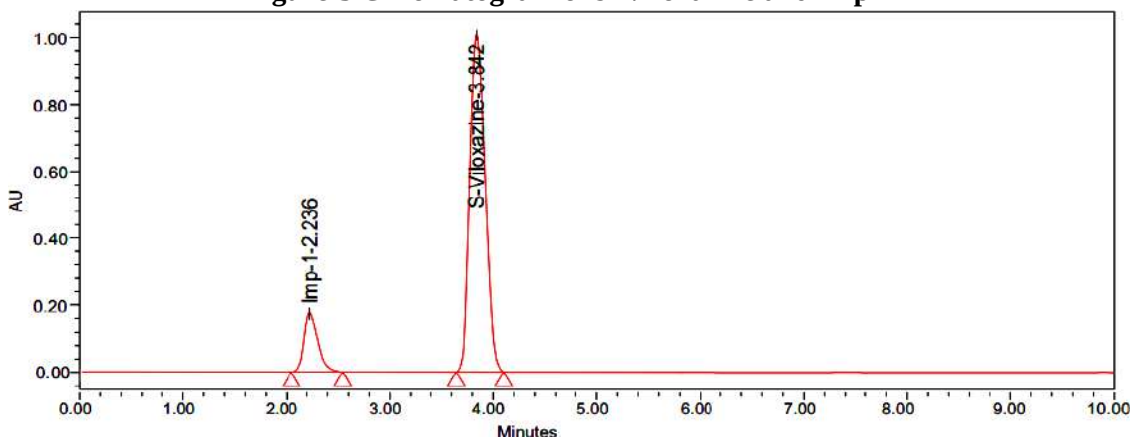


Figure-3 Chromatogram of s- Viloxazine and Imp-1



Retention time of s- Viloxazine Imp-1 and s-Viloxazine were about 2.236 and 3.842 min respectively.

METHODVALIDATION

1 System suitability:

System suitability done by injecting spiked standard solution six times into HPLC system. It was observed that all parameters were within the limits.

Table-2 Results (n=6) of System Suitability

Parameter	Imp-1	s-Viloxazine
Retention Time	2.236	3.842
Theoretical Plates	12639	20854
Tailing Factor	0.93	1.02
USP Resolution	---	4.21
%RSD ofpeakareas	0.61	0.75

2. Linearity and Range

For s-Viloxazine and Imp-1, the linearity approach was proven throughout concentration ranges of 125-750µg/ml and 1.25-7.5µg/ml, respectively. The aforementioned solutions were made as aliquots from stock solution and labeled 1, 2, 3, 4, 5, and 6 accordingly. The solutions were then injected into the HPLC system in accordance with the test protocol. Plotting the calibration curve for s- Viloxazine and Imp-1 in accordance with concentration Vs average peak area was done. Below are the findings.

Figure-4 Calibration curve of s- Viloxazine

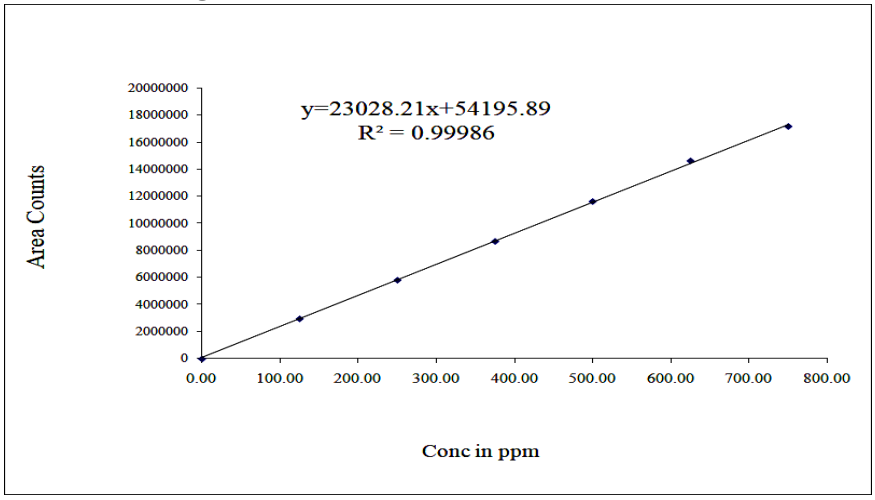


Figure-5 Calibration curve of Imp-1

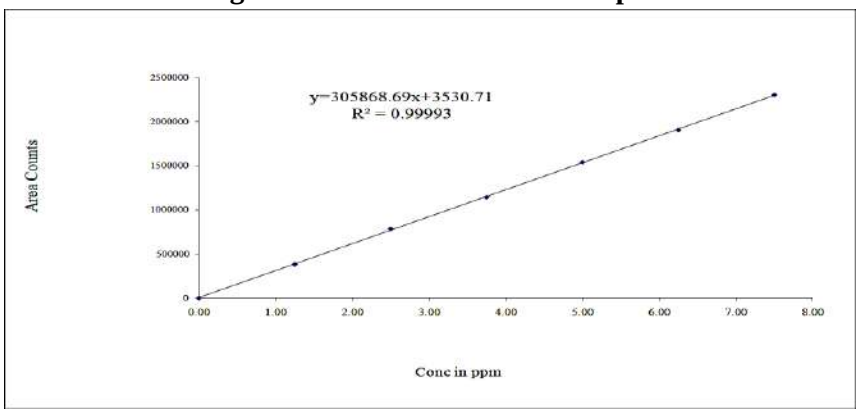


Table-3 Linearity Parameters for s- Viloxazine, Imp-1 (n=6)

Parameters	s- Viloxazine	Imp-1
Linearity range	125-750µg/ml	1.25-7.5µg/ml
Correlation coefficient	0.99986	0.99993

3. Precision:

Six injections from the same standard preparations were made for repeatability study, and the relative standard deviation for the replicate injections was computed. The system precision additions were listed below.

Table No: 4 Precision values for s- Viloxazine (n=6)

S.NO	Name of precision	Drug			Imp-1		
		mean	STD dev.	%RSD	mean	STD dev.	%RSD
1.	System Precision	11683477	87062.466	0.75	1542681	9429.472	0.61
2.	Method Precision	11606383	97974.178	0.84	1535398	10044.447	0.65

From the method precision and system precision studies, it was observed that all the parameters like %RSD of retention time and peak area were within the limits.

4. Accuracy:

A study of accuracy for s- Viloxazine and Imp-1 was conducted in triplicate (50%, 100%, and 150%) using the same amount of drug containing s- Viloxazine and Imp-1 into each volumetric flask for each spike level. A percentage recovery average was computed.

Table-5 Accuracy Results

DRUG	RECOVERY LEVEL	TARGET CONC in mg	AMOUNT TAKEN in mg	%MEAN RECOVERY	%RSD
s- viloxazine	50%	50	25	100.6	0.18
	100%	50	50	100.3	0.97
	150%	50	75	100.4	0.70
IMP- 1	50%	10	05	99.4	0.14
	100%	10	10	100.3	0.78
	150%	10	15	99.2	0.24

5. SPECIFICITY

A.s-Viloxazine and Imp-1 Identification

Solutions of the standard and the sample were prepared as per test procedure and injected into the system.

Figure-6 Chromatogram of s-ViloxazineandImp-1 (Standard)

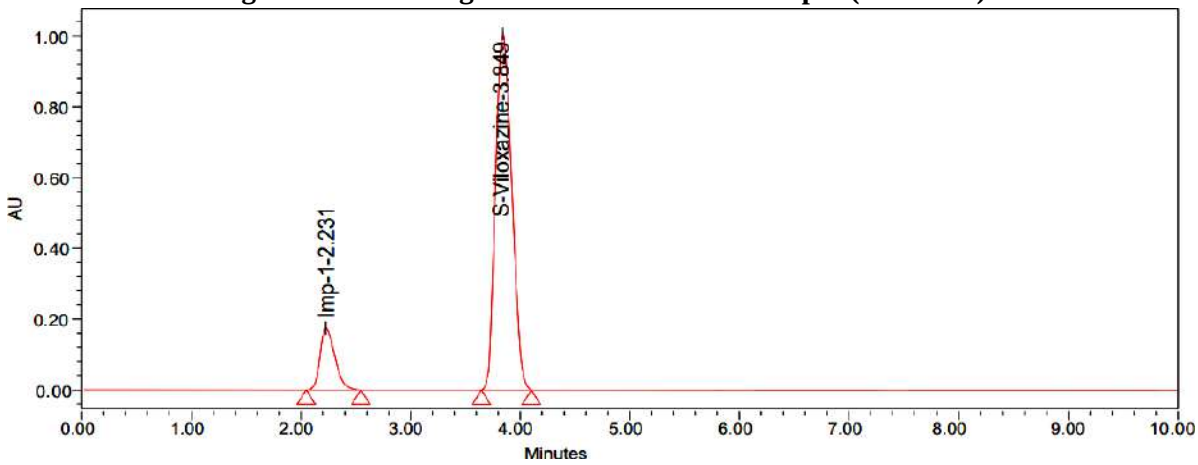
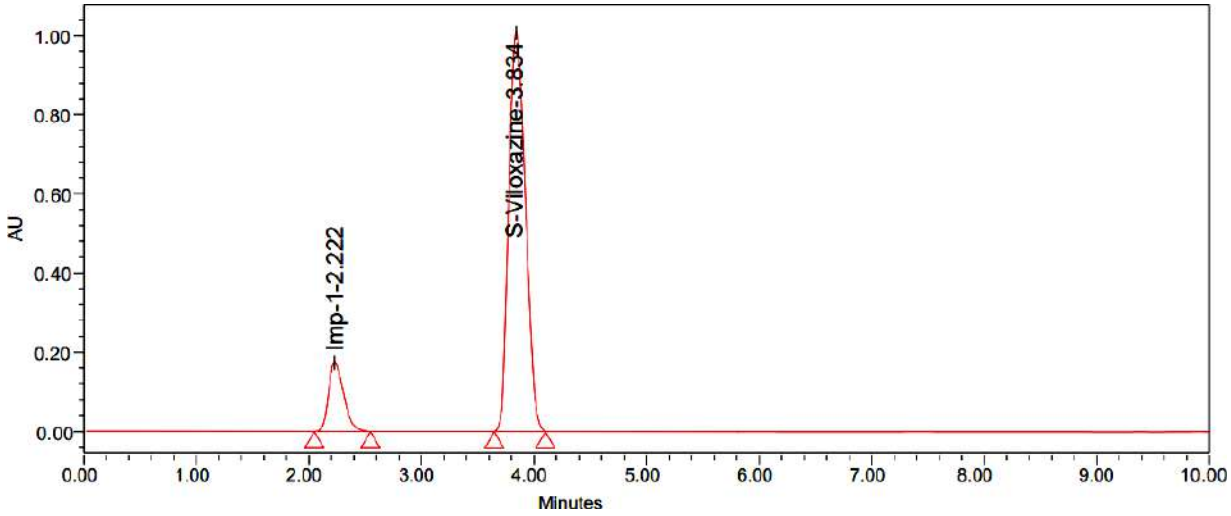


Figure -7 Chromatogram of s-Viloxazine and Imp-1(Sample)

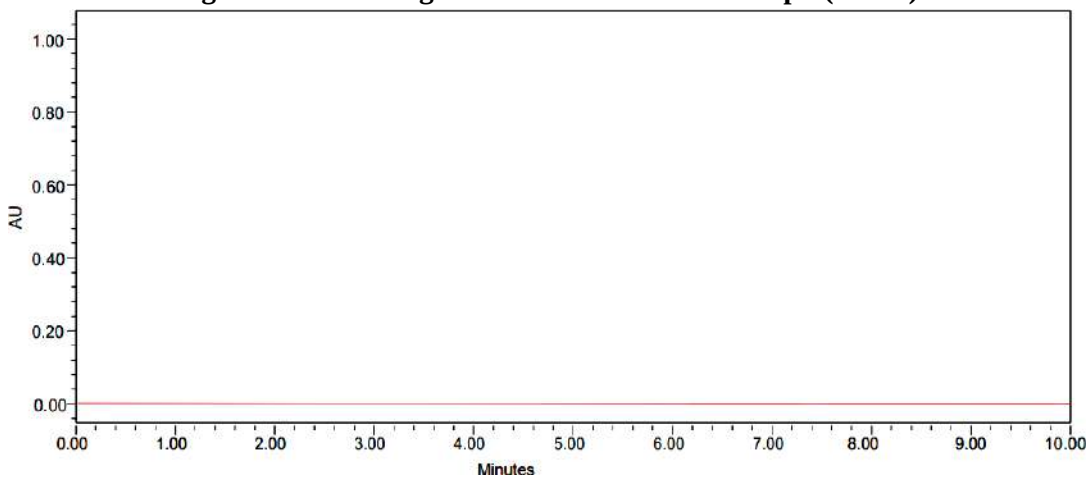


The chromatograms of the standard and sample were identical.

B. Blank Interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Figure-8 Chromatogram of s- Viloxazine and Imp-1(Blank)



There was no interference due to blank at the retention time of the analyte. Hence the method was specific.

6. Robustness

a. Effect of variation of flow rate:

Preparing a standard solution, it was injected into the HPLC at flow rates of 0.9 and 1.1 ml/min (±0.1 ml/min) to assess the impact. Below is an observation of the flow rate variation.

Table No: 6 Effect of variation of flow rate

DRUG	FLOW RATE in ml/min	PEAK AREA	RT in mins	%RSD
s- Viloxazine	0.9	10761145	3.984	0.66
	1.1	12417765	3.697	0.73
IMP-1	0.9	1426347	2.419	0.86
	1.1	1722540	2.158	0.65

Effect of variation of Organic Composition:

By altering the ratio of the mobile phase, or methanol and n-hexane and IPA, from 30:50:20 v/v to 27:55:18 v/v and 33:45:22 v/v, a study was carried out to ascertain the impact of modification in the mobile phase composition. After preparing and injecting the standard into the HPLC apparatus, the chromatograms were recorded.

Table No: 7 Effect of variation of Organic Composition:

DRUG	COMPOSITION	PEAK AREA	RT in mins	%RSD
s- Viloxazine	More Organic	13662487	3.545	**0.53**
	Less Organic	9962205	4.125	**0.17**
IMP-1	More Organic	1865243	2.032	**0.69**
	Less Organic	1266308	2.565	**1.12**

7. RUGGEDNESS:

Analyst to Analyst Variability

The study on analyst-to-analyst variability was carried out at various dates and by several analysts in comparable settings. According to the test procedure, two samples were prepared and each was examined. Chromatograms and the data were displayed in the table below.

Table- 8 Ruggedness data (Effect of changes in the analyst)

Analyst	Peak area of s- Viloxazine	Peak area of Imp-1
Analyst1	11669874	1537542
Analyst2	11592536	1544697
Mean	11631205	1541120
SD	54686.224	5059.349
%RSD	0.47	0.33

7. LODAND LOQ

The LOD and LOQ were calculated as per formula and were shown in the below table.

Table-9 Limit of Detection and Limit of Quantification

Sample	LOD(S/N)	LOQ (S/N)
Viloxazine (S-isomer)	3 µg/ml	10 µg/ml

Figure-10 Purity Plot of Control degradation

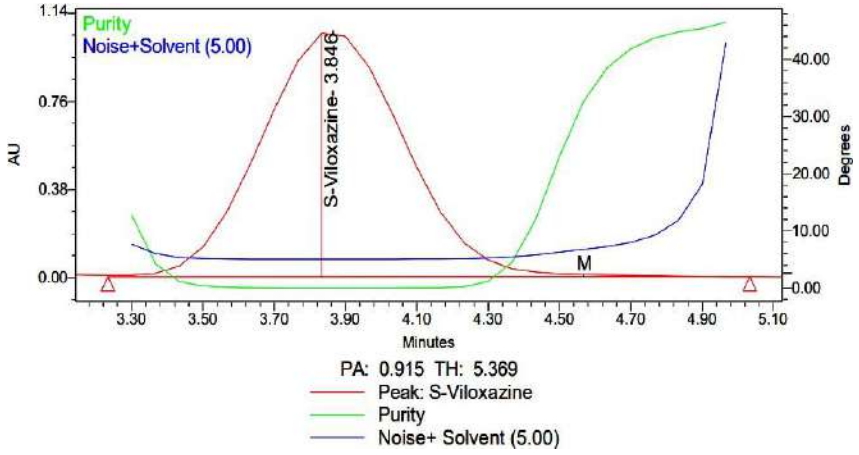


Figure - 11 Representative chromatogram for Acid degradation

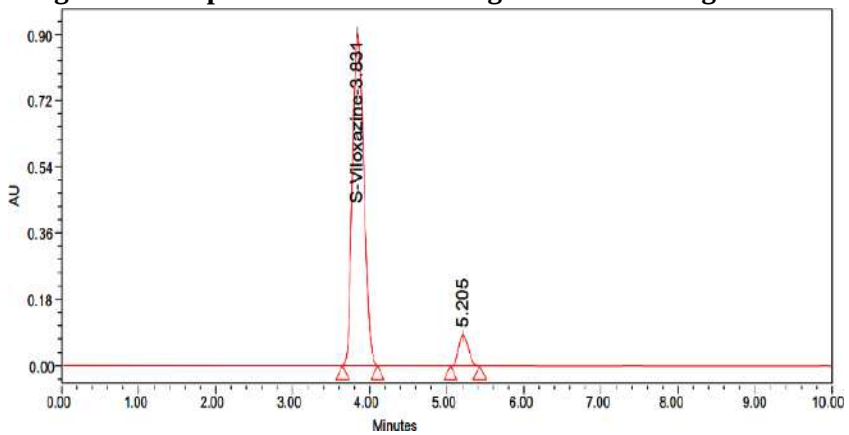


Figure-12 Purity Plot of Acid degradation

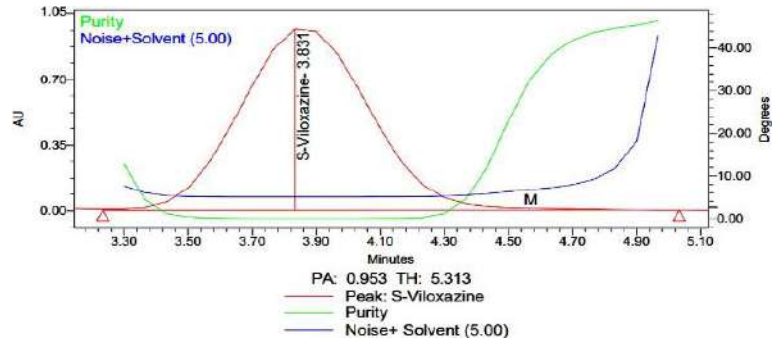


Figure-13 Representative chromatogram for Alkali degradation

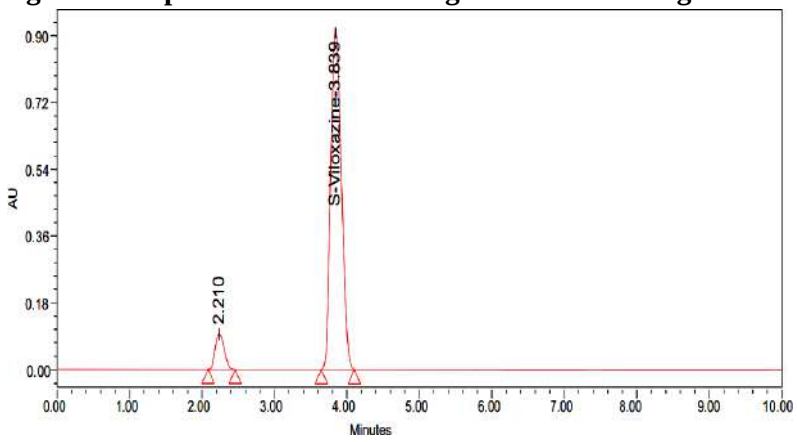


Figure -14 Purity Plot of Alkali degradation

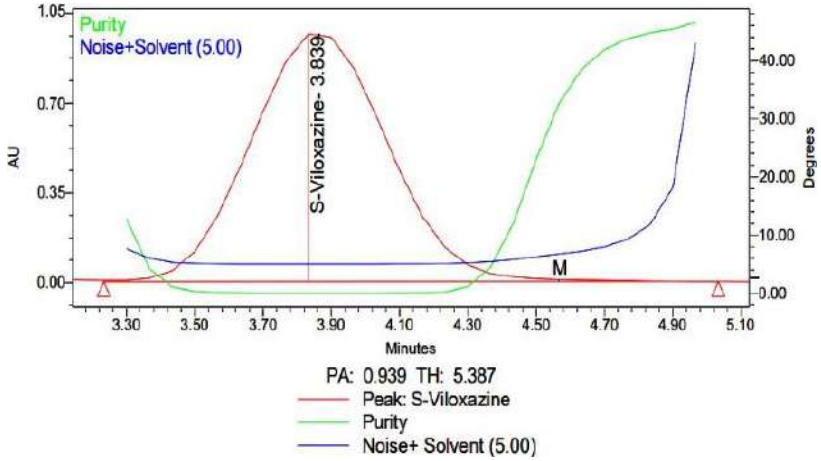


Figure-15 Representative chromatogram for Peroxide degradation

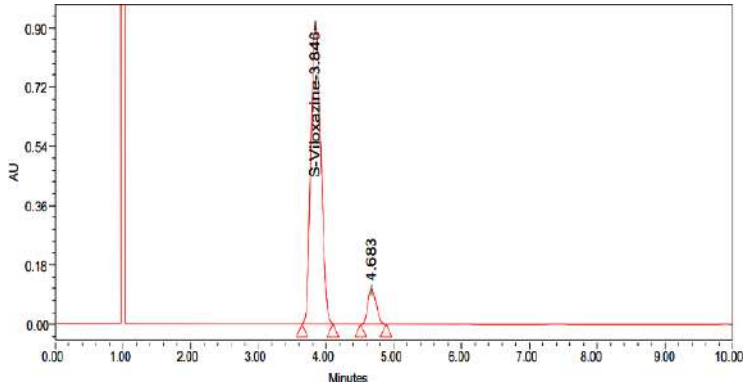


Figure-16 Purity Plot of Peroxide degradation

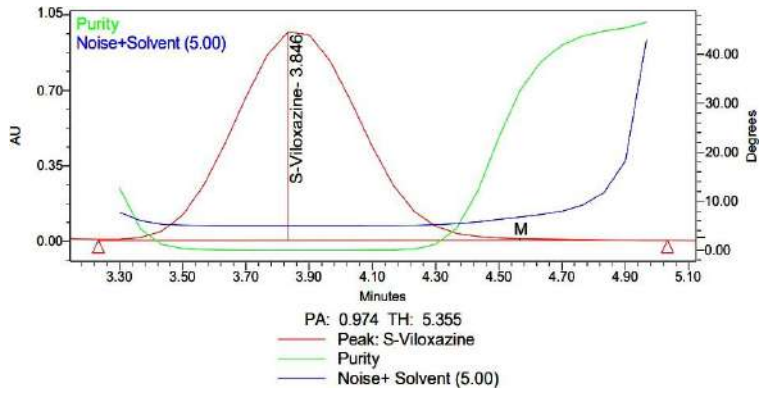


Figure-17 Representative chromatogram for Hydrolysis degradation

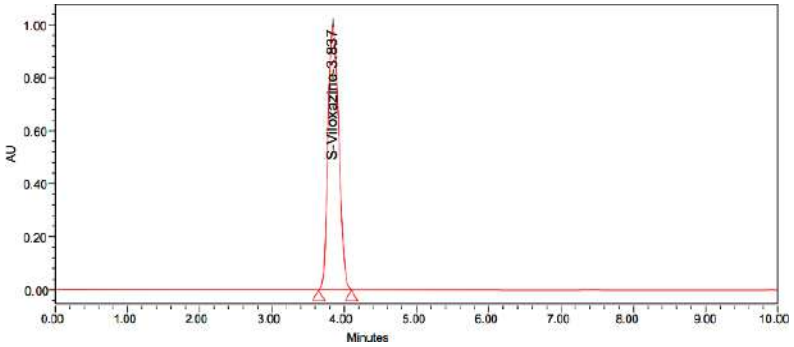


Figure-18 Purity Plot of Hydrolysis degradation

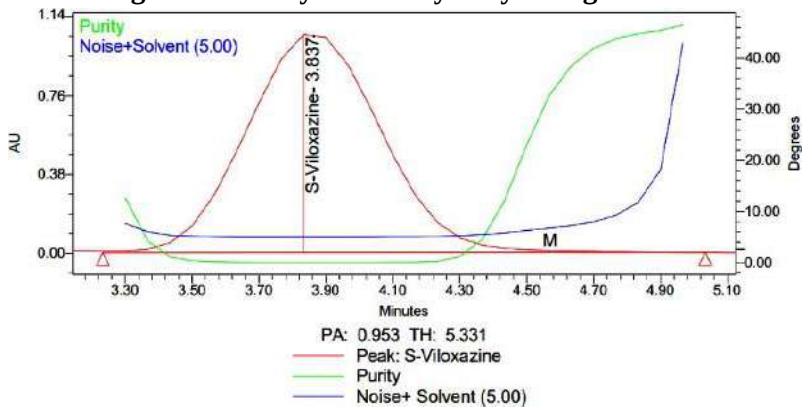


Figure-19 Representative chromatogram for Reduction degradation

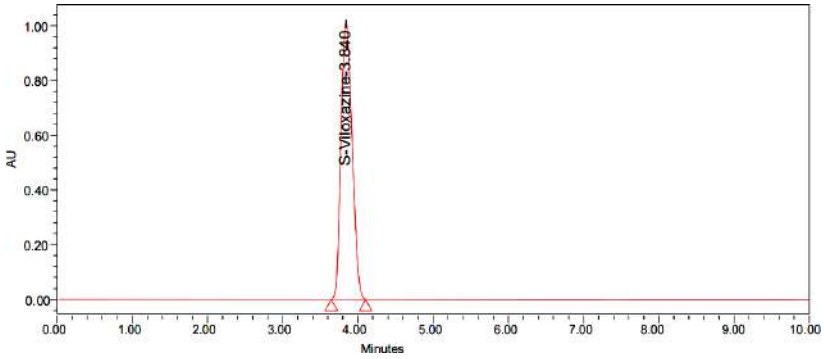


Figure-20 Purity Plot of Reduction degradation

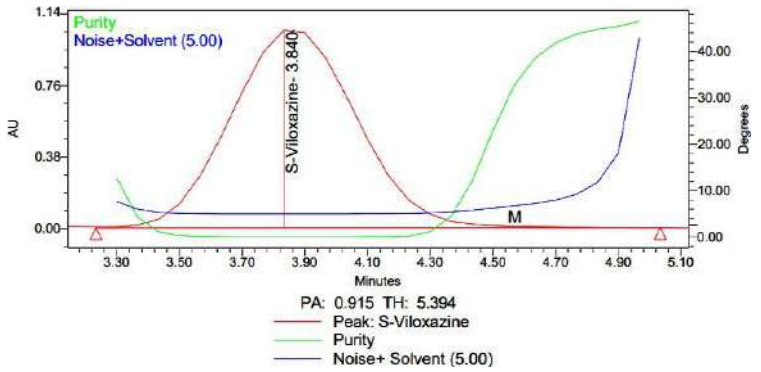


Figure- 21 representative chromatogram for photolytic degradation

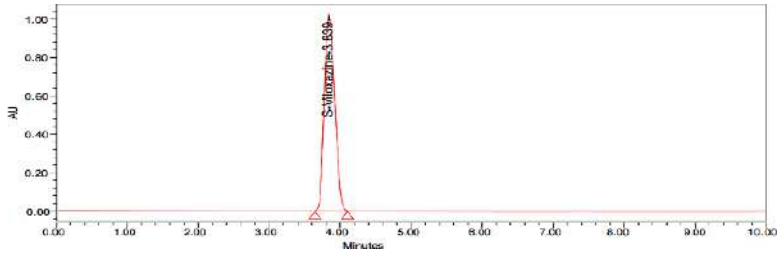
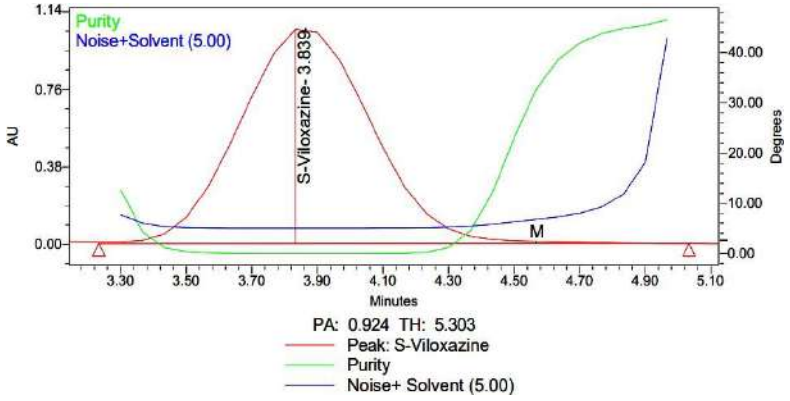


Figure-22 Purity Plot of Photolytic degradation



Figure– 23 Representative chromatogram for Thermal degradation

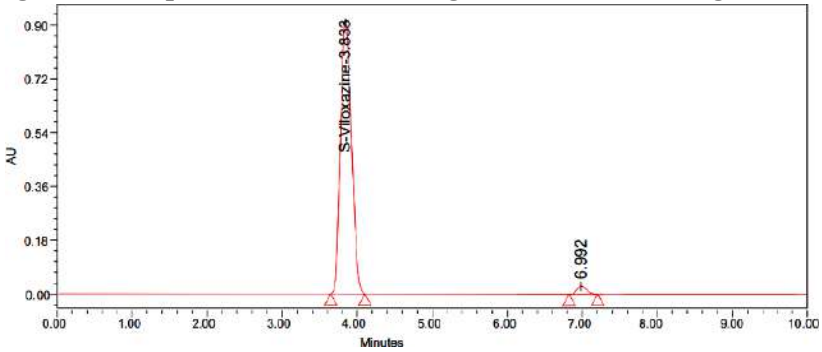


Figure-24 Purity Plot of Thermal degradation

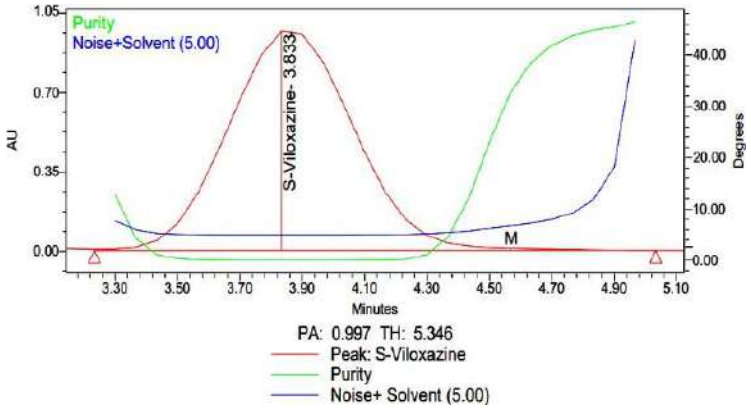


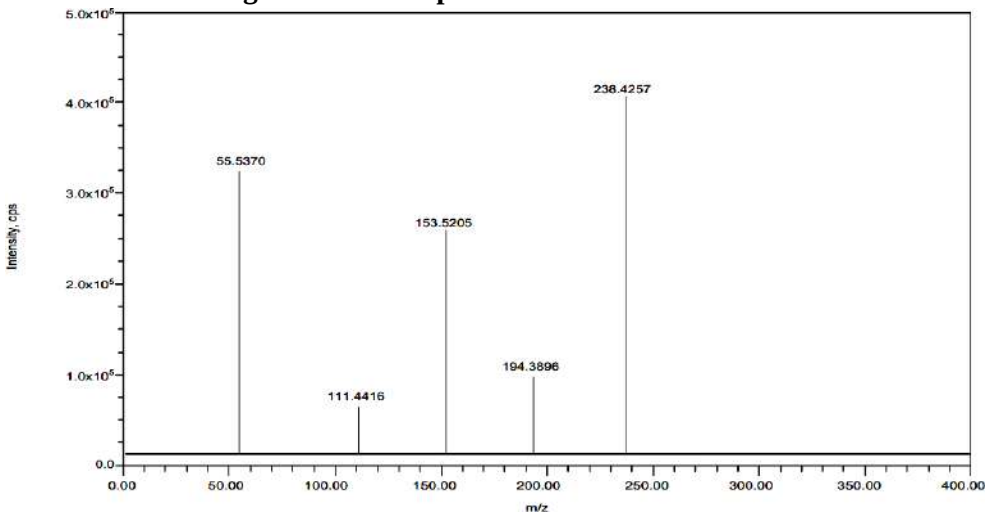
Table-10 Results of Forced Degradation study for s- Viloxazine

	% Assay after degradation	Purity Angle	Purity Threshold	%Degradation
Control	100	0.915	5.369	0
Acid	87.6	0.953	5.313	12.4
Alkali	86.9	0.939	5.387	13.1
Peroxide	85.4	0.974	5.355	14.6
Reduction	96.5	0.915	5.394	3.5
Thermal	88.9	0.997	5.346	11.1
Photolytic	95.8	0.924	5.303	4.2
Hydrolysis	97.7	0.953	5.331	2.3

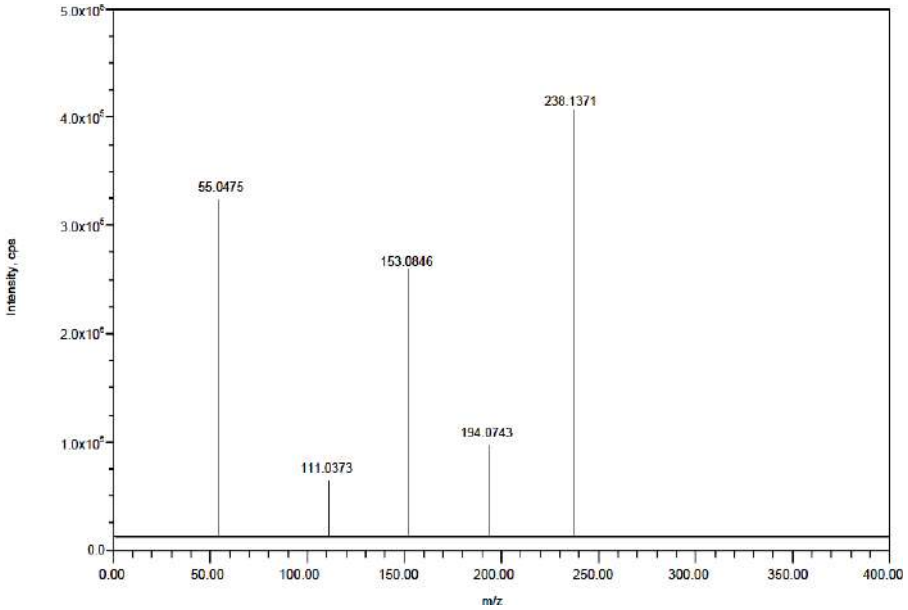
Purity angle is found to be less than threshold angle in all forced degradation studies without having signs of purity flags. Hence the proposed method was said to be stability indicating.

MS characterization:

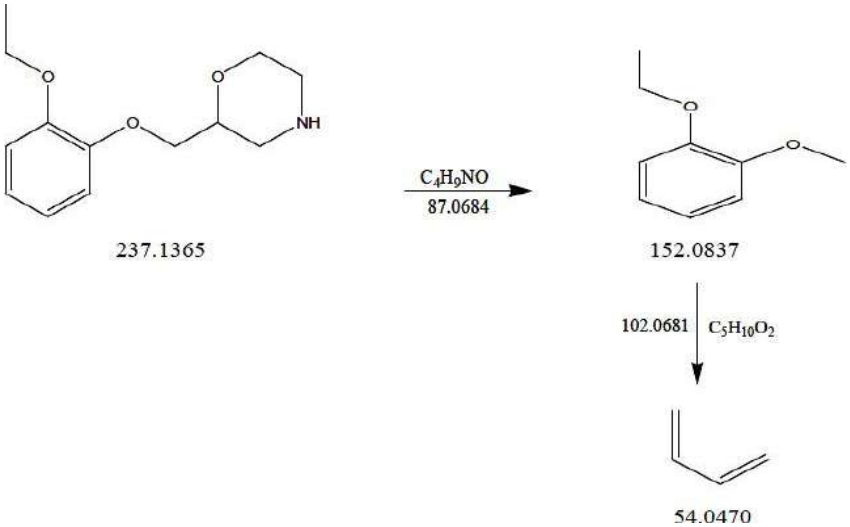
FigNo.25: Mass Spectra of Racemic Mixture



FigNo.26: Mass Spectra of S-Viloxazine

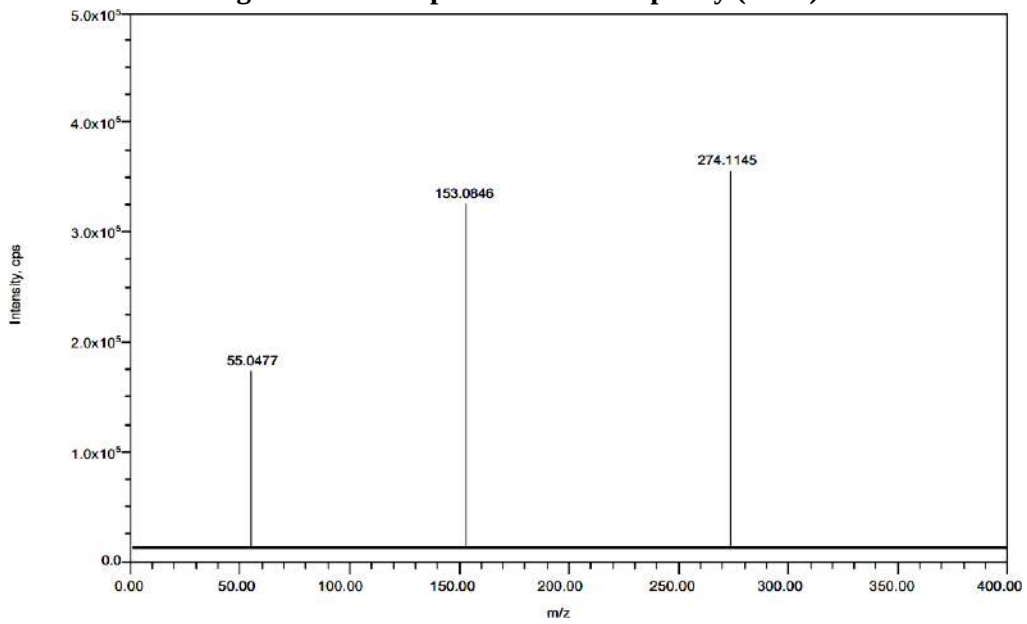


Collision –induced dissociation of S-Viloxazine: S-Viloxazine: The fragmentation mechanism of S-Viloxazine was depicted in Scheme 1, and the most strong [M+H]+ ion was detected at m/z-237.1365 in the ESI spectrum. The MS/MS spectra of S-Viloxazine revealed abundant productions at m/z-54.0470 (loss of C5H10O2 from m/z 152.0837) and m/z-152.0837 (loss of C4H9NO from m/z-237.1365). The suggested scheme has been validated by the MS/MS tests in conjunction with precise mass measurements.



FigNo.27: Fragmentation pathway of S-Viloxazine

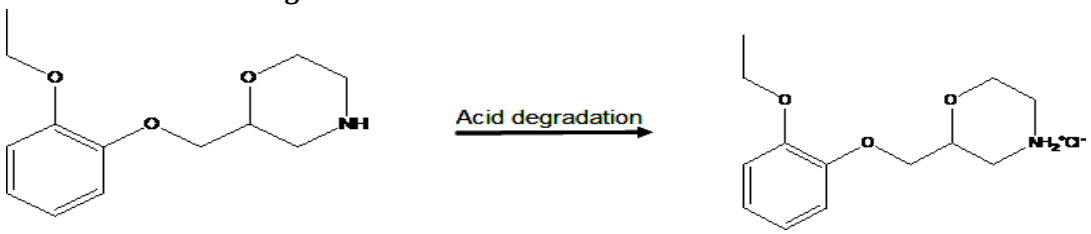
FigNo.28: Mass Spectra of Acid Impurity (DP-1)



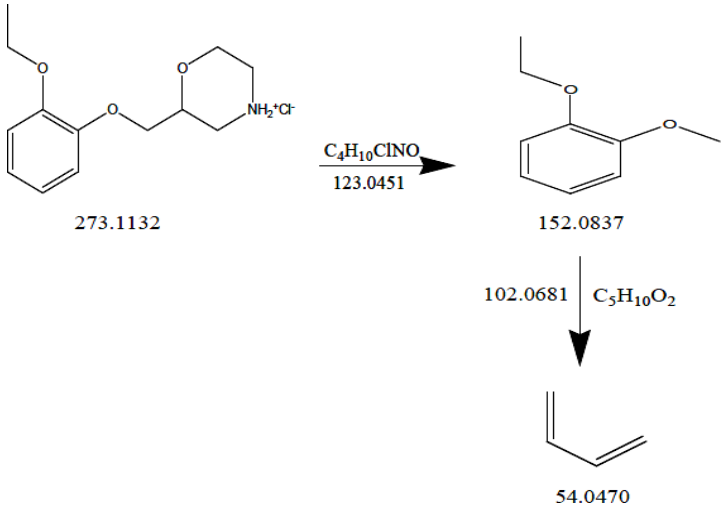
DP1:

The ESI spectra revealed the most strong [M+H]+ion of m/z-273.1132, which was detected under an acid degradation condition. Scheme 2 illustrates the fragmentation mechanism of DP1. At m/z-152.0837 (loss of C4H10ClNO from m/z-273.1132) and m/z-54.0470 (loss of C5H10O2 from m/z 152.0837), DP1's MS/MS spectra showed abundant product ions. The suggested scheme has been validated by the MS/MS tests in conjunction with precise mass measurements.

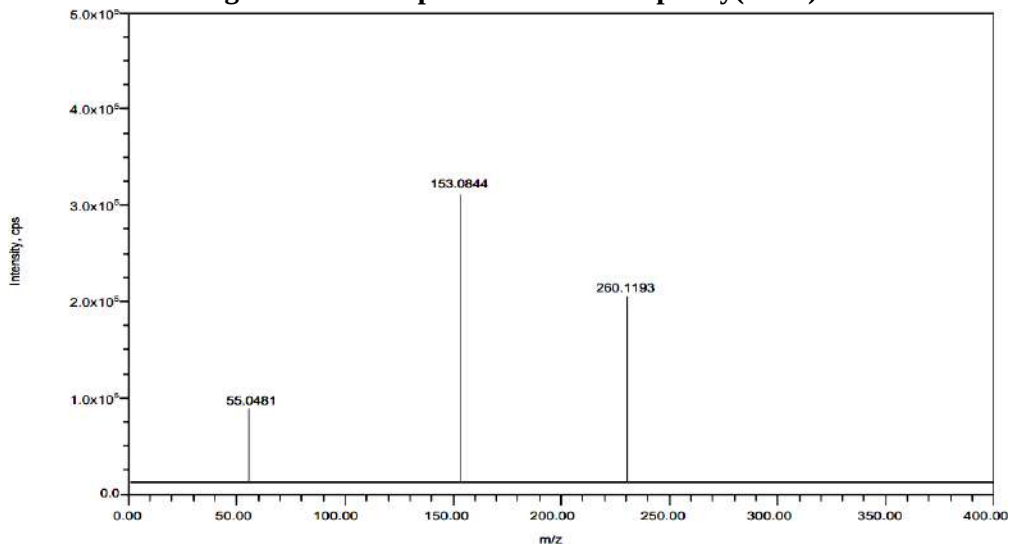
Fig No. 29: Reaction of S-Viloxazine with HCl



FigNo.30: Fragmentation path way of Acid Impurity (DP-1)



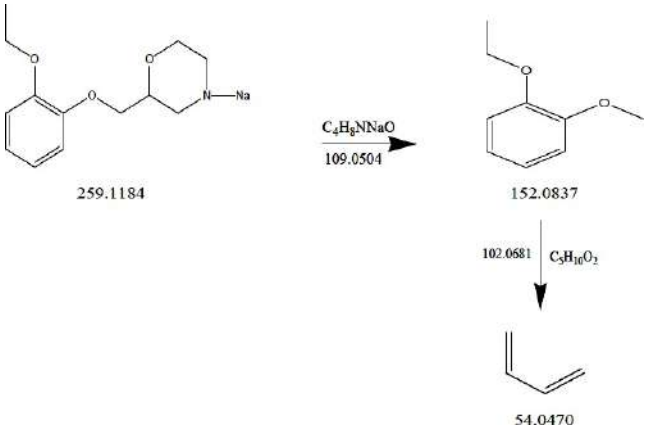
FigNo.31: Mass Spectra of Alkali Impurity(DP-2)



DP2:

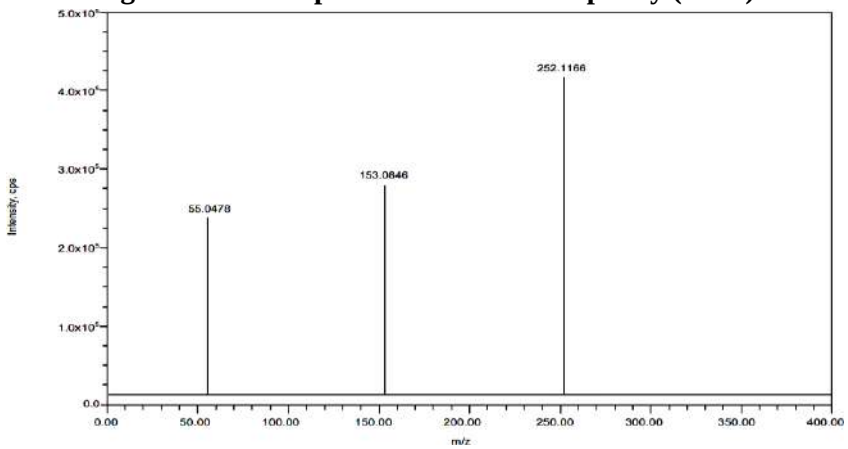
The ESI spectra revealed the most strong [M+H]+ion of m/z-259.1184, which was detected under alkali degradation conditions. Scheme 3 illustrates the fragmentation mechanism of DP2. Abundant product ions were seen in the DP2 MS/MS spectrum at m/z-152.0837 (loss of C4H8NNaO from m/z-259.1184) and m/z-54.0470 (loss of C5H10O2 from m/z 152.0837). The suggested scheme has been validated by the MS/MS tests in conjunction with precise mass measurements.

FigNo.32: Reaction of S-Viloxazine with NaOH



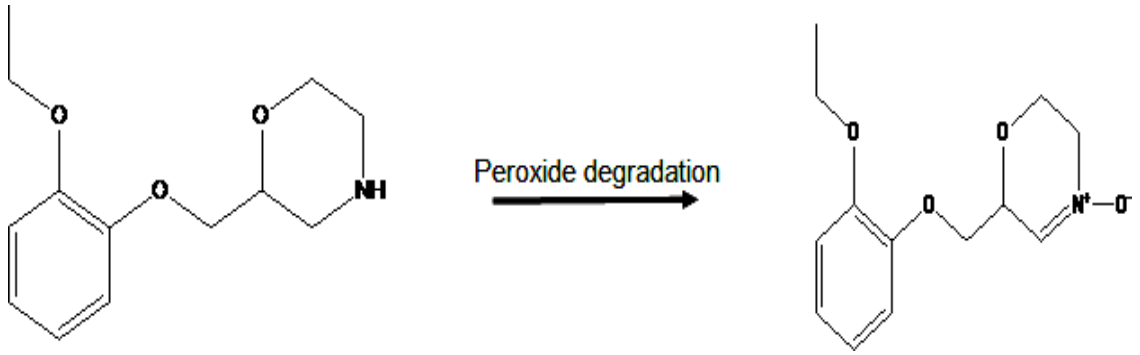
FigNo.33: Fragmentation pathway of Alkali Impurity(DP-2)

FigNo.34: Mass Spectra of Peroxide Impurity (DP-3)

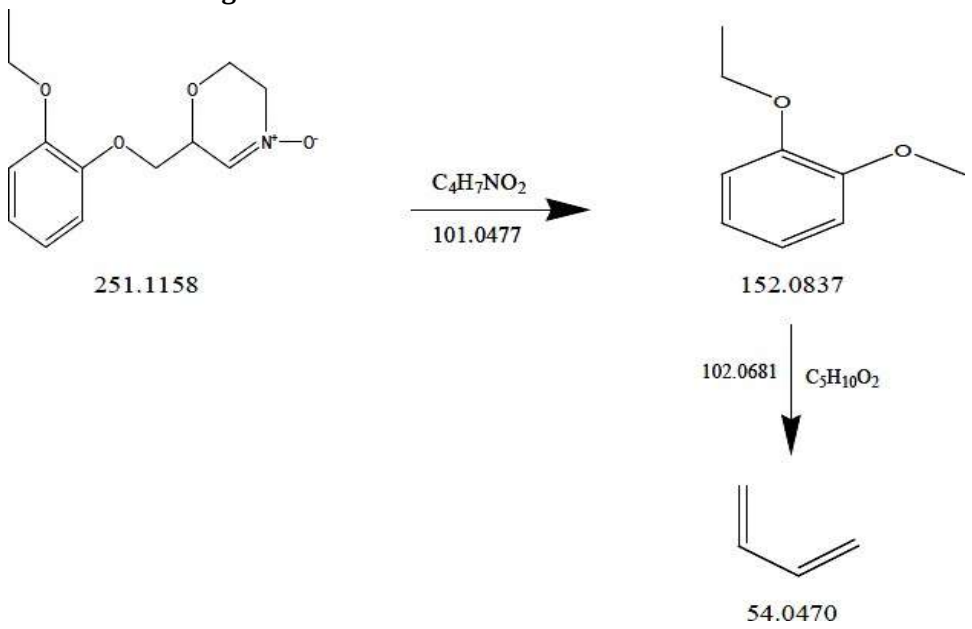


DP3:

The fragmentation mechanism of DP3 was illustrated in Scheme 4, and the most intense [M+H]+ ion of m/z-251.1158 was detected under the conditions of peroxide degradation, according to the ESI spectra. Abundant product ions were seen in the DP3 MS/MS spectra at m/z-152.0837 (loss of C4H7NO2 from m/z-251.1158) and m/z-54.0470 (loss of C5H10O2 from m/z 152.0837). The suggested scheme has been validated by the MS/MS tests in conjunction with precise mass measurements.

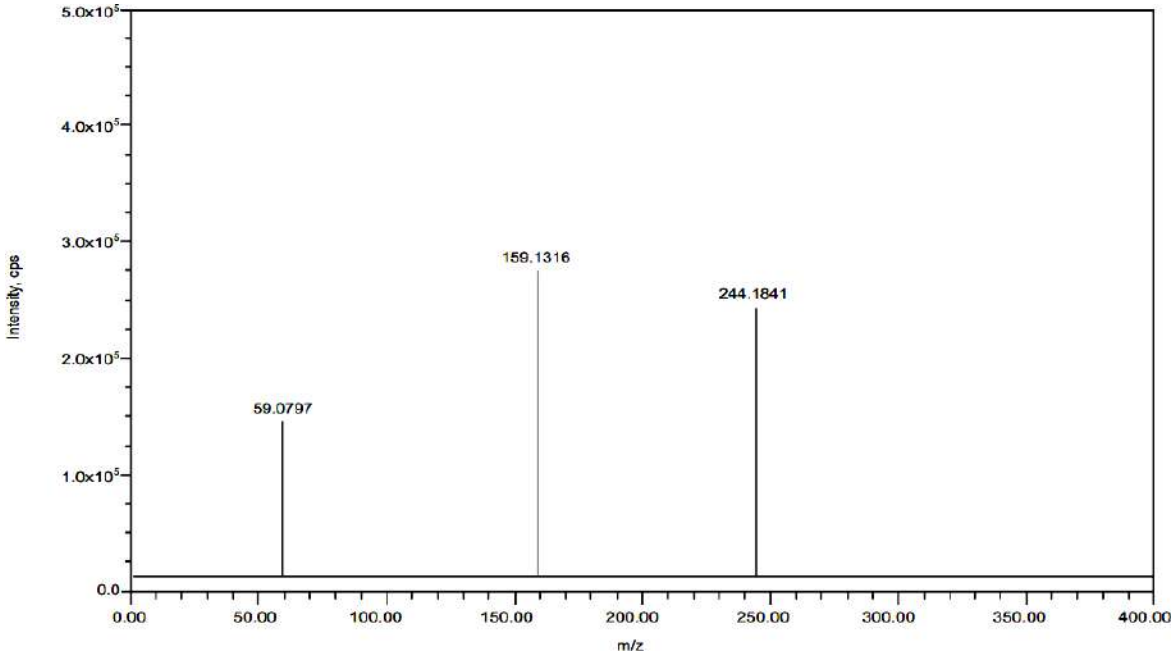


FigNo.35: Reaction of S-Viloxazine with H2O2



FigNo.36: Fragmentation pathway of Peroxide Impurity(DP-3)

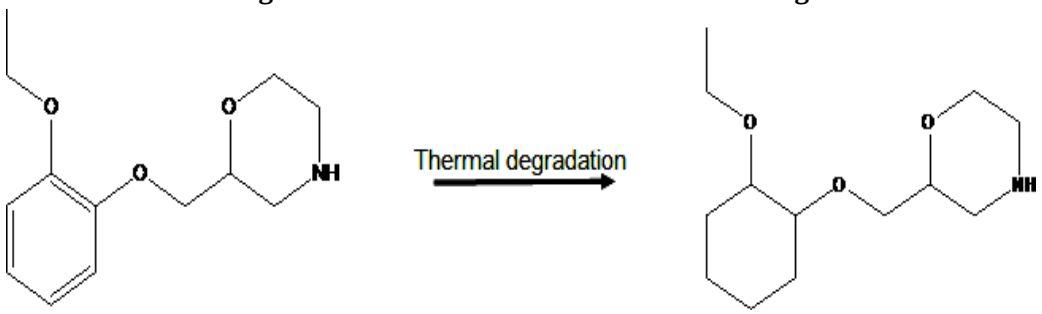
FigNo.37: Mass Spectra of Reduction Impurity(DP-4)



DP4:

The fragmentation mechanism of DP4 was depicted in Scheme 5, and the most intense [M+H]+ ion of m/z-243.1834 was detected under conditions of thermal deterioration, according to the ESI spectrum. The loss of C4H9NO from m/z-243.1834 and the loss of C5H12O2 from m/z 158.1307, as well as the abundance of product ions at m/z-58.0783, were evident in the DP4 MS/MS spectrum. All the suggested scheme have been validated by the MS/MS tests in conjunction with precise mass measurements.

FigNo.38: Reaction of S-Viloxazine on heating



FigNo.39: Fragmentation pathway of Reduction Impurity(DP-4)

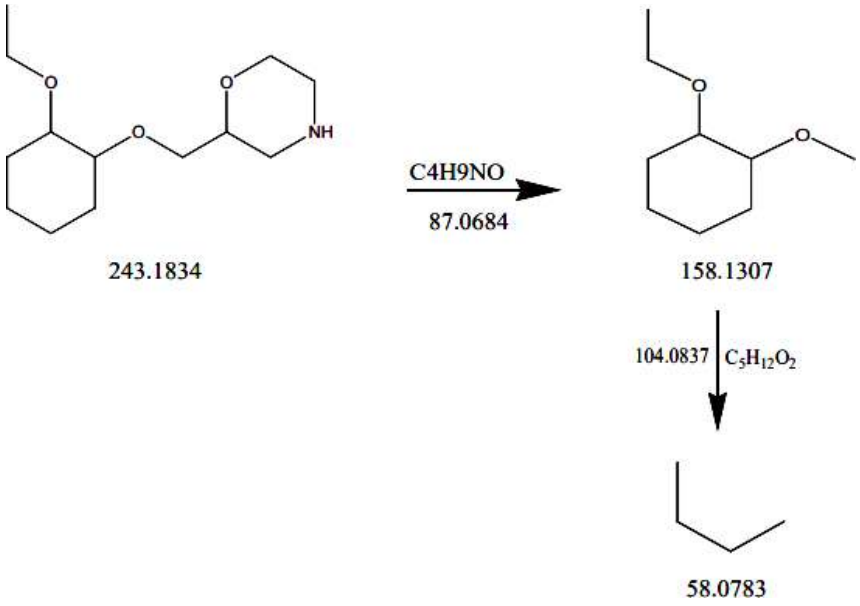


Table 11: Mass Spectrometric characterization Data:

Molecular Formula	Calculated Mass	Observed Mass	Error	Major Fragment Ions	Molecular Formula
S-Viloxazine	C13H19NO3	237.1365	237.1371	2.530188	55.0475, 111.0373, 153.0846and 194.0743
DP1	$C_{13}H_{20}ClNO_3$	273.1132	273.1145	4.759931	55.0477and 153.0846
DP2	$C_{13}H_{18}NNaO_3$	259.1184	259.1193	3.473316	55.0481and 153.0844
DP3	C13H17NO4	251.1158	251.1166	3.185781	55.0478and 153.0846
DP4	C13H25NO3	243.1834	243.1841	2.878486	59.0797and 159.1316

CONCLUSION:

The current study came to the conclusion that the chiral-HPLC stability indicating assay method was precise, accurate, and specific, and that it did not interfere with the placebo or degradation products. Therefore, routine analyses of s-Viloxazine and its impurities can be performed using this proposed method.

REFERENCES

1. Alisa R.Kosheleffetal., Population Pharmacokinetics of ViloxazineCapsules in Pediatric Subjects With Attention Deficit/Hyperactivity Extended-ReleaseDisorder:NationalLibraryOfMedicine,JCl inPharmacol.2021Dec;61(12).
2. Anitha Kethipalli, Mannam Krishnamurthy et al., Stability indicating and cost effectiveanalytical method development and validation of Viloxazine by using reverse phase highperformance liquid chromatography (2021): Journal of Cardiovascular Disease Research,VOL12,ISSUE 04, 2021.
3. Fazio,Antonino;Crisafulli,etal.,ASensitiveGasChromatographicAssayfortheDeterminationof Serum Viloxazine ConcentrationUsingaNitrogen-Phosphorus-SelectiveDetector:TherDrugMonit.1984;6(4):484-8.
4. M.Martinez, C.Sánchezdela Torre, E.Almarza,Simultaneous determination of viloxazine, venlafaxine, imipramine, desipramine, sertraline, and amoxapine in wholeblood:comparisonoftwoextraction/cleanupproceduresforcapillarygaschromatographywithnitrogen-phosphorusdetection:JAnalToxicol. 2002Jul-Aug; 26(5):296-302.

HOW TO CITE: Shaik Mastanamma , Chunduri Akhila , M. S. Dhandapani , Chiral HPLC Method Development And Validation For The Estimation Of S-Viloxazine And Fd Characterization By MS, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 8, 3697-3716. https://doi.org/10.5281/zenodo.13371310